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54 Name of Invention: Production Method For Water-soluble Keratin

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Specification

1. Name of Invention

   Production Method For Water-soluble Keratin Protein

2. Scope of Patent Application

   A method of producing water-soluble keratin protein through partial hydrolysis by means of acid or alkali, through enzymatic degradation, through oxidative degradation, or through reductive degradation, after keratin protein has been soaked in an solution of alkaline salts.

3. Detailed Description of Invention

   (Applicable industrial fields)

   This invention concerns a method, using hard protein keratin (referred to in the rest of this specification as "keratin protein") whose main ingredients are composed of substances such as the body hair, horns, nails and hoofs of cows and pigs, wool, feathers, and hair, which, after the performance of an alkali process, and by means of the subsequent performance of partial hydrolysis by means of acid or alkali, enzymatic degradation, oxidative degradation, or reductive degradation, produces keratin protein having a proposed desired molecular weight. Keratin protein

produced using the method of this invention can be used in food products, cosmetics and industrial products.

(Conventional art)

Up to now, various methods to solubilize keratin protein have been proposed by many researchers.

Basically, the method used to date consist of two processes in which the disulfide bond (-S-S-) that exist in the cystine residue of the keratin protein is split with a thiol group (-SH) reduction agent (the first process), and subsequently, the peptide bond principal chain is cut using enzymes in a liquid medium (the second process). In the first process of this method, the keratin protein is first swollen by the addition of a urea solution, and subsequently a mercaptan such as thioglycolic acid, mecaptoethanol, thioglycerin, and thiosalicylic acid, or a sulfide such as sodium sulfide, potassium sulfide, calcium sulfide, triethanolamine sulfide, diethanolamine sulfide, and monoethanolamine sulfide, is used as a reducing agent by which the disulfide bond is cut. In the second process, in general acidic enzymes like pepsin are used in the pH 1-3 range, and neutral enzymes like bromelain are used in the pH 5-8 range to sever the peptide bonds. The method which comes from this second process forms the heart of the research of this present water-soluble keratin protein production method.

(Problem that the invention proposes to solve)

However, in the existing method, there is a characteristic problem in their construction.

In the second process of the existing method, the ease with which the peptides are cut is controlled by the conditions of the first process. Consequently, because anything that affecting the details or conditions of the first process become a major problem, this company is carrying out various investigations. However, since there is a limitation that, in order for the reducing agents in the first process to work efficiently, the pH must be maintained in the alkali range, the investigation of these conditions is not entirely easy.

Therefore, since the management of the first process and the second process that uses enzymes is complex, controlling the reaction is relatively difficult. There is also the problem that each process requires long times, and both are expensive. Also, because there is a large loss for each process of the existing method, there is also a problem with poor yields.

(Means by which this problem is solved)

This invention is a method that offers to solve the problems of the existing method, producing a water-soluble keratin protein of a desired molecular mass appropriate for the purposes of food products, cosmetics and industrial use products, as well as shortening processing time, and obtaining good yields with a simpler process.

The type of keratin, to which this invention applies, includes any hair, horns, nails, and hoofs of cows and pigs, wool, feathers, or hair made up of keratin protein.

This invention is fundamentally a method that includes the alkali processing and partial degradation of the second process of the existing method. Therefore, this invention's principal characteristic is, given that the keratin protein is put through an alkali process, it departs from the existing method with a fundamentally different mechanism, producing water-soluble keratin protein.

This invention's alkali process is carried out by soaking the keratin protein in an alkaline salt solution. Although there are many alkaline salts that can be used for the solution in this invention, among those, the use of calcium hydrochloride, sodium hydrochloride, or potassium hydrochloride is preferred. However, in general, calcium hydrochloride is particularly preferred.

By soaking the keratin protein in this alkaline salt solution, the disulfide bonds (– S – S –) in the keratin molecule partially change to thioether bonds (– S –). Differing from the existing method which severs the disulfide bonds and makes a thiol group (– S H), this invention has the novel characteristic that thioether bonds are partially formed in the keratin protein. More specifically, it is a characteristic in which the cystine residue, which possesses the disulfide bonds in the keratin protein, is changed to lanthionine residue that possesses thioether bonds. Because the thioether bonds are extremely stable, they are not severed in the peptide degradation process that takes place after the alkali processing, remaining in the keratin protein until the end. Therefore, by controlling the lanthionine generation during the alkali processing, it is possible to regulate the molecular weight of water-soluble keratin protein finally produced. Control of lanthionine residue generation is done by changing any one of the conditions of alkaline salt solution concentration, alkali processing time and temperature, or by changing a combination of these conditions. More specifically, if calcium hydroxide is used as an alkaline salt, a concentration from 0.1% by weight to a saturated liquid (3-4% by weight), a pH in the range of 11-13, a processing temperature of less than 40° C, and a processing time of less than 24 hours, all of which can be modified, can be used. Also, if sodium hydroxide or potassium hydroxide are used, a concentration of 0.001-0.1%, a pH range of 11-13, a processing temperature of less than 40° C, and a processing time of less than 24 hours, all of which can be modified, can be used. The alkaline salt solution may be agitated during the alkali process. Also, before and after the alkali processing, the keratin protein is washed water, as is suitable in the normal process.

Concerning the keratin protein targeted by the process, when the relation between the condition of the alkali process and amount of lanthionine residue generated is clearly done, good yield of water-soluble keratin protein of the desired molecular weight can be obtained. For example, in test example 1, concerning the keratin used, if the alkali process time is lengthened, the conversion rate of cystine residue to lanthionine residue rises, this definite relationship is shown in Table 1. By making clear this relationship, with regards to processing temperature and other conditions, it becomes possible to accurately select the conditions needed to obtain water-soluble keratin having the desired molecular weight. Consequently, in combination with the conditions of the peptide decomposition after the alkali processing, it becomes possible to accurately regulate the molecular weight. When comparing this invention with the previous method in this way, because there are many conditions that can be

changed, this invention also has the characteristic of being able to regulate molecular weight with higher accuracy.

In the alkali process of this test example, it also becomes clear that, outside cystine residue and lanthionine residue, no other amino acids are substantially changed. Therefore, this invention's alkali process selectively acts on cystine residue, and is not accompanied by undesired side reactions. Furthermore, because this invention's alkali process, or soaking the keratin protein in an alkaline salt solution is extremely simple and easy, and the processing time is also short, its utility is exceedingly valuable.

After the keratin protein has been alkali processed, the partial decomposition of the peptide bonds is dealt with. This partial degradation can use existing methods normally used for acid hydrolysis, alkali hydrolysis, enzymatic degradation, oxidative degradation, and residual degradation. In comparison to the existing method described above, in which acid or alkali partial degradation cannot be performed in order to keep amino acid levels needed for decomposition to proceed, this invention has a wide choice of methods of peptide degradation. For example, if acid hydrolysis is used with this invention, 1-2 kg of keratin protein is added in ratio to 4-8 kg of 10-30% by weight hydrochloric acid at 80-100° C for 1-10 hours to perform the degradation. After partial degradation takes place, deoxidation is done using an anionic exchange resin. Or, for example, if alkali hydrolysis is used, 1-2 kg or keratin protein is added in ratio to 4-8 kg of an aqueous solution that is 0.1-10% sodium hydroxide at 70-100° C for 1-5 hours to perform the degradation. After partial degradation takes place, deoxidation is done using a cationic exchange resin.

This invention's method of producing water-soluble keratin protein may include processes outside of the alkali processing and peptide partial degradation described above. For example, purifications like desalinization, filtration, deodorizing and bleaching may be performed after the peptide partial degradation. In addition, after partial degradation and purification, concentration and drying may be done. Consequently, an antiseptic may be added to the solution.

Although this invention is concretely described in the following examples of execution and investigation test examples, the scope of this invention is not limited to these examples of execution and test examples.

Example of Execution 1

After washing 1 kg of keratin protein in water, it was soaked in an aqueous solution of 1% calcium hydroxide by weight, for 6 hours. After that, the keratin protein was washed with water, and added to 4 liters of an acidic solution that was 30% hydrochloric acid by weight and boiled at 100° C for 2 hours. This solution was bleached with activated carbon, deodorized, and a pale yellow-brown oligo-keratin was obtained. The molecular weight of the oligo-keratin obtained was measured by a gel filtration method and consequently proved to be 1000.

Example of Execution 2

After washing 1 kg of keratin protein in water, it was soaked in an aqueous solution of 1% calcium hydroxide by weight, for 2 hours. After that, the keratin protein was washed with water, and added to 4 liters of an acidic solution that was 10%

hydrochloric acid by weight and boiled at 100° C for 4 hours. This solution was bleached with activated carbon, deodorized, and a pale yellow-brown oligo-keratin was obtained. The molecular weight of the oligo-keratin obtained was measured by a gel filtration method and consequently proved to be 400.

Example of Execution 3

After washing 1 kg of keratin protein in water, it was soaked in an aqueous solution of 0.5% calcium hydroxide by weight, for 24 hours. After that, the keratin protein was washed with water, and added to 5 liters of 50% performic acid at 35° C for 24 hours using oxidative degradation. This solution was bleached with activated carbon, deodorized, and a pale yellow-brown oligo-keratin was obtained. The molecular weight of the oligo-keratin obtained was measured by a gel filtration method and consequently proved to be 1000.

Test Example 1

After washing the keratin protein in water, it was soaked in an aqueous solution with a final concentration of 0.5% calcium hydroxide by weight, at room temperature. The composition of cystine residue, lanthionine residue and other amino acid residues in the keratin protein were measured for keratin protein not soaked in the solution, and keratin proteins that were washed in water after being removed from the solution 1, 6 and 10 hours after soaking began. These results are shown in Chart 1.

Chart 1

Amino Acid	Amino Acid Concentrations from Alkali Processing Test				
	Not Soaked	1 Hour	2 Hours	6 Hours	24 Hours
Cysteinic Acid	5.8	5.6	4.8	7.7	6.7
Asparaginic Acid	61.5	62.3	61.1	62.8	61.3
Threonine	72.2	71.3	69.8	72.6	70.5
Serine	111.6	110.4	106.9	109.8	105.1
Glutamic Acid	125.8	129.5	127.8	129.0	125.1
Proline	75.7	75.6	73.9	75.5	75.8
Lanthinine	2.3	24.3	36.9	34.5	63.1
Glycine	79.0	78.8	76.4	79.6	77.2
Alanine	53.2	53.1	52.8	53.1	53.3
Cystine	70.9	58.3	52.8	48.8	34.1
Valine	57.1	57.7	56.3	57.1	57.5
Methionine	4.5	4.2	4.5	4.6	4.8

Isoleucine	33.3	31.9	33.3	32.7	33.0
Leucine	73.0	71.1	71.6	72.1	71.7
Tyrocine	29.9	19.0	19.5	19.2	22.1
Phenylalanine	24.7	22.2	25.1	22.1	22.1
Lysine	33.0	37.5	38.9	31.0	27.7
Histidine	14.9	14.2	14.7	9.5	14.5
Arginine	73.0	73.0	72.3	78.6	74.5

Test Example 2

After washing keratin protein in water, it was soaked for 1, 6 and 24 hours in an aqueous solution that was 2% calcium hydroxide by weight, at 28° C. Next, 1 kg of the keratin protein, after it was washed in water and neutralized, was put in 8 liters of an aqueous solution that was 2.9% sodium hydroxide by weight, at 80° C for 3 hours, and was hydrolyzed. After the keratin protein was hydrolyzed, and after it was purified and desalinated, it was examined at the ultraviolet wavelength of 280 nm while maintaining the Sephandex G-75 column, measuring the change in molecular weight. Diagram is a chromatogram of each of the samples soaked for 1, 6 and 10 hours. Each sample was compared to the standard calibration curves of ovalbumin, bovine serum albumin and cytochrome C, and the peak molecular weights of the samples soaked for 1, 6 and 10 hours were estimated to be 9,700, 19,000 and 36,000 respectively. According to this test experiment, it is shown that the longer the time spent soaking in the alkaline salts, the more lanthionine residue is produced, and larger the molecular weight finally generated becomes.

Simple Explanation of Diagram

Diagram 1 is a chromatograph from the Sephadex G-75 of the alkali hydrolyzed keratin protein after soaked in a solution of calcium hydroxide according to the conditions of Test Example 2.

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Diagram 1
Vertical Axis: Optical Density at 280nm
Horizontal Axis: Amount of elution (ml)
Tallest Curve: 24 hrs.
Middle Curve: 1 hr.
Shortest Curve: 6 hrs.
Three terms at bottom of diagram: bovine serum albumin (mw 68000); ovalbumin (mw 45000); Cytochrome C (mw 12500)